Pestic. Sci. 1998, **53**, 21–28

A General Joint Action Model for Herbicide Mixtures

Jens C. Streibig,[1]* Per Kudsk[2] & Jens E. Jensen[1]

[1] Department of Agricultural Sciences, Section of Weed Science, The Royal Veterinary and Agricultural University, Thorvaldsensvej 40, DK-1871 Frederiksberg C, Denmark
[2] Department of Crop Protection, Danish Institute of Agricultural Sciences, Flakkebjerg, DK-4200 Slagelse, Denmark

(Received 19 March 1997; revised version received 29 September 1997; accepted 5 January 1998)

Abstract: The assessment of mixture effects is usually done with isoboles which illustrate whether mixture effects are greater or smaller than would be expected on the basis of the individual activities of the herbicides. Under the assumption of similarity of response curves and by incorporating a function that can model the shape of isoboles, we can statistically test whether divergence from the Additive Dose Model (ADM) is significant. Two dose-response experiments with mixtures of either salt or ester formulations of MCPA and mecoprop-P and one experiment with tribenuron-methyl and mecoprop-P were analysed. Mixtures of tribenuron-methyl and a salt formulation of mecoprop-P showed antagonism. Mixtures of salt formulations of MCPA and mecoprop-P followed ADM, whilst ester formulations of the same compounds showed synergism. To get reliable estimates, the model requires mixture ratios covering the whole isobole

Pestic. Sci., **53**, 21–28 (1998)

Key words: Additive Dose Model; synergism; antagonism; phenoxyacids; tribenuron-methyl

1 INTRODUCTION

Mixtures of herbicides are used to control diverse weed floras with species of varying sensitivity, to delay development of resistant biotypes of weeds and to reduce cost of application. Mixtures include tank mixtures, which are either pre-mixed by the manufacturer or mixed by the end user. The joint action of mixtures is not only confined to tank mixtures but can, in some instances, be seen with sequential spraying.

The literature reveals a Babylonian confusion of mixture models, in which additivity of herbicide effects and additivity of doses are confused. Traditionally, some mixture research is based on empirical studies at some pre-set dose rates in factorial designs and sometimes analysed with polynomial regressions.[1,2] In factorial designs with mixtures of, for example, two herbicides, the interaction is based upon the effects of the herbicides and merely tells us whether an effect of a herbicide remains unchanged in mixture with another herbicide. Interaction will inevitably occur if the dose range is wide enough, because at very low and very high doses the responses approach the upper and lower limit of the dose response curve. Consequently, such interactions are of little biological relevance. A more general way to describe the joint action of herbicide mixtures is to use the response curves of the herbicides applied alone and in mixtures and incorporate various joint action reference models, for example the Additive Dose Model (ADM) or the Multiplicative Survival Model (MSM).[3–5]

The ADM assumes that, at a defined response level, the effect of a mixture of two herbicides can be expressed by the relative potency of the two herbicides applied separately. If we assume that 90% weed control is achieved by either spraying 1 kg ha^{-1} of mecoprop or 0·004 kg ha^{-1} of tribenuron-methyl, then we can

* To whom correspondence should be addressed.
Contract/grant sponsor: Ministry of Food, Agriculture and Fisheries, Research Secretariat.

 Pestic. Sci. 0031-613X/98/$17.50. Printed in Great Britain

mix the two herbicides in any proportion without changing the 90% weed control by using their relative potency of 250 (1/0·004). For example if we want to make a mixture with 0·500 kg ha^{-1} of mecoprop then we must add 0·002 kg ha^{-1} of tribenuron-methyl (0·500/250) to get a mixture that still gives 90% control of the weeds. It means that spraying 0·502 kg ha^{-1} of this mixture will, according to ADM. still yield a control level of 90%. ADM is analogous to the exchange of currencies, the exchange rate between currencies resembling that of relative potency between herbicides.

The MSM assumes that a herbicide in a mixture affects the plant independently of the other herbicide in the mixture, that is the herbicides have entirely different modes of action. MSM further assumes that the plant response can be expressed as a proportion of a hypothetical maximum value. It was developed for quantal responses (dead or alive) and not for graded response as is used here. If we use the example from above with 1 kg ha^{-1} of mecoprop and 0·004 kg ha^{-1} of tribenuron-methyl, then a mixture of 1 kg ha^{-1} of mecoprop and 0·004 kg ha^{-1} of tribenuron-methyl will yield 99% weed control [1-(1-0·9)(1-0·9)]: the effects are multiplicative.

The results of experiments using herbicides in mixtures are commonly presented graphically with ADM or MSM isoboles which are contours of a set of mixtures giving the same effect, e.g. 50% effect (ED_{50}).[4–6] If the observed mixture points deviate from an ADM isobole, the mixture is either more effective (synergism) or less effective (antagonism) than expected from the effects of the herbicides applied separately. Gessner[7] gives a comprehensive review of how to assess graphically deviations of observed mixture points from ADM isoboles. A statistical test for the deviation of herbicide mixtures from ADM has been given elsewhere.[5] If herbicide mixtures deviate from ADM, the question arises whether it is possible to determine not only a significant deviation from ADM but also the magnitude of this deviation based upon the data. In essence, the simple ADM isobole is rarely justified by the available data. If ADM is rejected on the basis of statistical tests we have not, to date, been able to satisfactorily describe and test alternative isoboles. Recently, Vølund[8] suggested a model that is a generalization of the ADM and which can describe various degrees of synergism and antagonism. This model may be an attractive alternative to graphical presentation of isoboles because it can be used as a predictive tool. When we know the shape of an isobole we can predict the behaviour of mixtures not included in the experimental design. Whether Vølund's model is capable of defining the shape of isoboles requires experience with various compounds and carefully designed experiments.

The aim of this paper is to incorporate the model by Vølund[8] into a logistic dose-response model to quantify additive action (ADM), reduced action (antagonism) and enhanced action (synergism) of mixtures. The hypothesis is that Vølund's model can be used to quantify deviations of mixtures from ADM and hence can be used to predict the effect of any mixture ratio.

2 MATERIALS AND METHODS

Two experiments were conducted in a greenhouse. Either *Veronica persica* Poir or *Sinapis alba* L. was grown in 2-litre pots containing soil + sand + peat (2 + 1 + 1 by weight). The experimental design was a complete randomized design with six doses and three replicates within each dose response curve. The untreated controls were replicated six times (Table 1). The pots were automatically sub-irrigated and the night temperature was kept above 8°C.

After emergence, *V. persica* was thinned to six plants per pot and sprayed at the four-true-leaf stage (Hardi 4110-14 nozzle at 157 litre ha^{-1}) with tribenuron-methyl and mecoprop-P (formulated as a potassium salt) either alone or in four mixtures with a fixed ratio between the herbicides (Table 1). Nineteen days after spraying, the fresh weight of plants in each pot was measured.

After emergence, *S. alba* was thinned to four plants per pot and sprayed at the 2–2·5-true-leaf stage (Hardi 4110-14 nozzle at 190 litre ha^{-1}) with MCPA as a dimethylamine salt or a butoxyethyl ester, mecoprop-P as a potassium salt or an ethylene glycol diester, either alone or in five mixtures with a fixed ratio between the herbicides (Table 1). Twenty-one days after spraying, the fresh weight of plants in each pot was measured.

TABLE 1
Mixtures and Maximum Dose Rates

%	*Herbicide*	*Maximum dose* (*g AI* ha^{-1})
	Veronica persica	
100	Mecoprop-P	1200
100	Tribenuron-methyl	3
99.5	Mecoprop-P	600
98	Mecoprop-P	150
92	Mecoprop-P	40
75	Mecoprop-P	15
	Sinapis alba	
100	MCPA	128
100	Mecoprop-P	128
80	MCPA	160
67	MCPA	192
50	MCPA	128
33	MCPA	192
20	MCPA	160

The dose range is 1/32, 1/16, 1/8, 1/4, 1/2 of the maximum dose plus the untreated control of zero dose.

2.1 Dose-response models

The response of fresh weight, (U) on dose, (z) was assumed to be well described by the logistic model:[9,10]

$$U_{ij} = C + \frac{D - C}{1 + \exp[b_i(\log(z_{ij}) - \log(ED_{50(i)}))]}, \quad (1)$$

where U_{ij} denotes the fresh weight at the jth dose of the ith herbicide mixture; D and C denote the upper and lower limit of fresh weight at zero and at infinite doses and were assumed to be the same for all reponse curves within an experiment. $ED_{50(i)}$ denotes the dose required of herbicide i to reduce fresh weight by half between the upper and lower limit, D and C; and b_i is proportional to the slope of the curve around $ED_{50(i)}$. A special case of eqn (1) is when the response curves within an assay have similar D, C and b parameters, i.e. the curves are similar, also called parallel or, in the nonlinear case, generalized parallel;[9,10] then eqn (1) can be reduced to:

$$U_{ij} = C + \frac{D - C}{1 + \exp[b(\log(r_i z_{ij}) - \log(ED_{50(1)}))]}. \quad (2)$$

The curves have the same D, C, b, $ED_{50(1)}$ parameters and their horizontal displacement relative to the standard herbicide is r_i for the ith herbicide mixture. The subscript (1) denotes the standard herbicide, which, by definition, has $r_1 = 1.00$. The advantage of reducing eqn (1) to eqn (2) is that, apart from reducing the number of parameters, we can easily test various hypotheses of the joint action of mixtures because the relative potency, r_i, which is the ratio of biologically equivalent dose of herbicides, is constant at any one response level. Theoretically, if the herbicides have the same site of action, then all other things being equal, their response curves should be similar with a relative horizontal displacement described by r_i.[11] On the other hand, the assumption of similar curves is a necessary but not a sufficient condition for assuming similar mode of action of compounds.[9]

2.2 Joint action models

The following description of joint action models only considers mixtures with two active ingredients. Any consistent model must relate the biological response of a mixture of doses to the biological response of the dose z_1 and z_2 of two compounds applied separately. Also, it must be reduced to a proper relation between response and z_1 as z_2 approaches zero in mixtures and *vice versa*. Finney,[12] Hewlett[13] and Hewlett and Plackett[14] have given a general introduction to the assessment of the joint action of mixtures of drugs. To a certain extent their terminology, together with that of Morse,[3] is used here.

The two reference models ADM and MSM were essentially developed to describe mixture effects in well-defined in-vitro systems. ADM assumes that doses of herbicides in a mixture can interchange with each other, based upon their relative potency, without changes in efficacy (see eqn (3)). This would usually hold for compounds having exactly the same site of action, e.g. for herbicides with exactly the same target site, all other things being equal. ADM can be applied to both quantal responses, e.g. dead or alive, and graded responses, e.g. biomass.

MSM assumes that the herbicides have independent modes of action in the plant. MSM was developed for quantal responses with a fixed maximum response of 1. This constraint complicates its use with graded responses in that maximum response of untreated control is also subject to experimental error and therefore not fixed. If the response curves have no lower limit ($C = 0$ in eqns (1) and (2)), the responses can be scaled by the upper limit, D in eqns (1) or (2), and then used for further analyses. But if there is a lower limit, different from zero, its use is more questionable.

Hewlett and Plackett[15] showed, for quantal responses, that ADM and MSM are special cases of a general model for combined action. Recently, Dresher and Boedeker[16] and Kudsk and Mathiassen[17] compared ADM and MSM at low and high effect levels of the mixture. They demonstrated that the relationship between ADM and MSM depends on the slope of the curves and the mixture dose administered. For small doses both MSM and ADM predict virtually the same mixture response, provided that the lower limit, C, is zero. In complex systems, such as whole plants, neither ADM nor MSM may predict mixture responses satisfactorily, particularly if formulation ingredients or adjuvants are used to facilitate herbicide uptake. Also, deviation from ADM may occur if the two compounds interact with each other's absorption, translocation or binding at their site(s) of action.

Consider any given response level, e.g. ED_{50}. Assume that Z_1 and Z_2 are the corresponding doses of herbicide 1 and 2 when applied singly, and z_1 and z_2 are the doses of herbicide 1 and 2 in a mixture with the same biological response. The relative potency between herbicide 1 and 2 is $r = Z_1/Z_2$. Assuming ADM, the equivalent doses z_m are given by:

$$Z_1 = rZ_2 = z_m = z_1 + rz_2 . \quad (3)$$

The relative potency r gives the 'biological' exchange rate between the herbicides when applied alone.

As shown elsewhere,[3,5] the right hand side or eqn (3) can substitute $r_i z_{ij}$ in eqn (2) to describe mutually parallel dose-response curves for the herbicides applied alone and in mixtures of fixed ratios. The ADM, therefore, can be tested by fitting response curves with eqn (3) for the herbicides applied alone and in mixtures of fixed ratios.[5] The magnitude of deviation and the shape of the isobole deviating from ADM, however, cannot be described by eqn (3). Hewlett[13] suggested a Joint Action Ratio, which implicitly assumes that the isoboles are

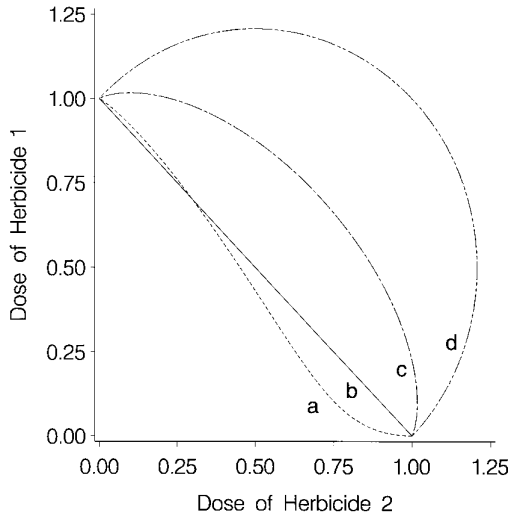

Fig. 1. Isoboles at 50% effect level from eqn (4). a is for $\eta_1 = 0{\cdot}5$ and $\eta_2 = 1{\cdot}5$; b is for $\eta_1 = \eta_2 = 1$; c is for $\eta_1 = \eta_2 = 1{\cdot}5$; d is for $\eta_1 = \eta_2 = 2$. The doses have been scaled so that the doses giving 50% effect are 1·00.

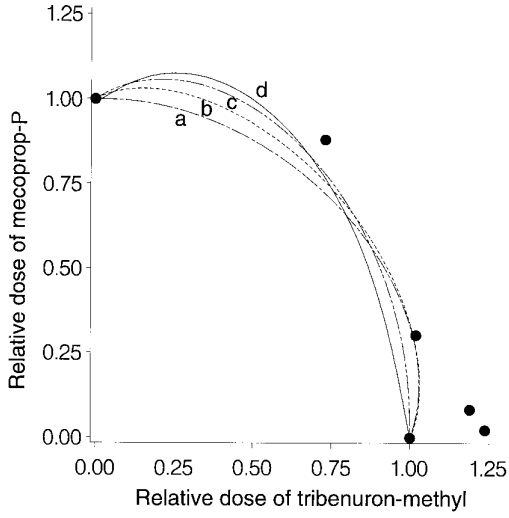

Fig. 2. Isoboles and data for mecoprop-P and tribenuron-methyl from Table 2. The doses have been scaled so that the doses of mecoprop-P and tribenuron-methyl applied separately are 1·00. (●) Mixture doses calculated from the parallel line regression in Table 2; a is for $\eta_1 = 1$ and $\eta_2 = 2{\cdot}41$; b is for $\eta_1 = \eta_2 = 1{\cdot}57$; c is for $\eta_1 = 2{\cdot}61$ and $\eta_2 = 1$; d is for $\eta_1 = 3{\cdot}47$ and $\eta_2 = 0{\cdot}78$.

symmetric,[18] but if the isobole is asymmetric the Joint Action Ratio falls short of being of predictive value. Another avenue to take is to let data determine the shape of the isobole, be it symmetrical or asymmetrical. This approach will make isoboles a more important predictor than the Joint Action Ratio and, even better, we can test the significance of the shape of the isobole.

Recently, Vølund[8] suggested a joint action model which can describe non-additivity of doses.

$$z_m = z_1^{\eta_1}(z_1 + rz_2)^{1-\eta_1} + (rz_2)^{\eta_2}(z_1 + rz_2)^{1-\eta_2}, \quad (4)$$

where z_m is the equivalent dose of the mixture. Figure 1 shows some isoboles calculated by eqn (4). If η_1 and η_2 are equal to 1·00, then eqn (4) reverts back to eqn (3) and the mixtures follow ADM (isobole marked b in Fig. 1). If η_1 and η_2 are similar but different from 1·00 we get symmetric isoboles (marked c and d in Fig. 1), otherwise we get asymmetric isoboles (marked a in Fig. 1). If η_1 and η_2 are smaller than 1·00, then the mixtures exert

TABLE 2
Summary of Regression Analyses of Mixtures of Salt Formulations of Mecoprop-P (Reference Herbicide) and Tribenuron-methyl Using *Veronica persica* as the Test Species

		Parallel curves		*Parallel curves and equation (4)*	
Mecoprop-P (%)	*Parameter*	*Estimate*	*Standard error*	*Estimate*[a]	*Standard error*
	D (g per pot)	98·0	3·53	98·0	3·48
	b	1·03	0·04	1·03	0·05
100	ED_{50} (g ha^{-1})	71·51	8·08	72·01	8·38
0	r_2	165·35	17·65	145·9	13·6
99·5	r_3	1·13	0·12		
98	r_4	3·25	0·35		
92	r_5	11·12	1·20		
75	r_6	33·40	3·70		
	η_1			3·48	2·48
	η_2			0·78	0·46
	$\eta_1 = \eta_2$			1·57	0·12
	η_1			2·61	0·46
	η_2			1	—
	η_1			1	—
	η_2			2·41	0·42

[a] Parameters when $\eta_1 \neq \eta_2$.

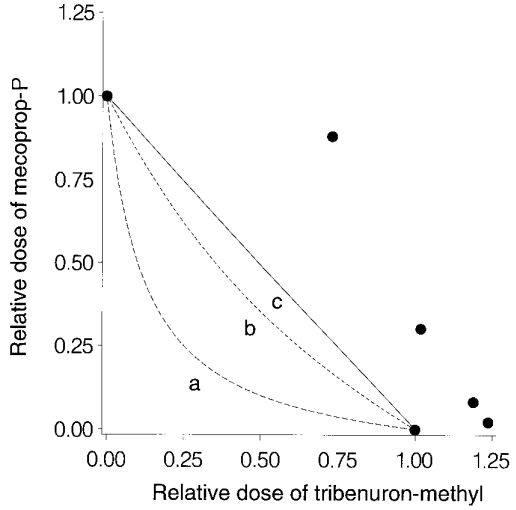

Fig. 3. MSM isoboles and data for mecoprop-P and tribenuron-methyl from Table 2. The doses have been scaled so that the doses of mecoprop-P and tribenuron-methyl applied separately are 1·00. (●) Mixture doses calculated from the parallel line regression in Table 2; a is for ED_{90}, b is for ED_{50} and c is for ED_{10}.

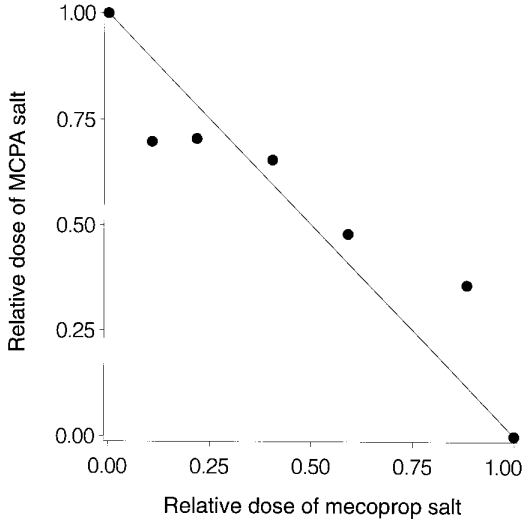

Fig. 4. Isoboles and data for salt formations of MCPA and mecoprop-P from Table 3. The doses have been scaled so that the doses of MCPA and mecoprop-P applied separately are 1·00. (●) Mixture doses calculated from the parallel line regression in Table 3.

enhanced effects (synergism) and if η_1 and η_2 are greater than 1·00, then the herbicides in mixtures are detracting from each other's action (antagonism) (isoboles marked c and d in Fig. 1). Equation (4) is for fixed mixture ratios, a monotone function and the parameters η_1 and η_2 are independent of the units of measurement of the dose. Equation (4) can be substituted for $r_i z_{ij}$ in eqn (2).

2.3 Statistical analyses

Within an experiment, the nonlinear regression models were fitted simultaneously to the response curves for the two herbicides applied alone and in mixtures. In order to stabilize the variance, a Transform-Both-Sides method[19] was used:

$$h(y, \lambda) = h(f_i(z), \lambda) + \sigma\varepsilon \tag{5}$$

With $h(\cdot)$ given by

$$h(x, \lambda) = \frac{x^\lambda - 1}{\lambda} \tag{6}$$

where $f_i(z)$ is the response model, either eqns (1), (2) or eqn (2) with eqn (4) incorporated, λ is the exponent of a power transformation in eqn (6) suggested by Box and Cox,[20] σ is the standard deviation and ε the residuals, corresponding to different observations, and are assumed to follow the standard normal distribution. The reduction of the regression model from eqn (1) to (2) and to (2) with eqn (4) incorporated was assessed by

TABLE 3
Summary of Regression Analyses of Mixtures of Salt Formulations of MCPA (Reference Herbicide) and Mecoprop-P Using *Sinapis alba* as the Test Species

		Parallel curves		*Parallel curves and ADM*	
MCPA (%)	*Parameter*	*Estimate*	*Standard error*	*Estimate*	*Standard error*
	D (g per pot)	57·3	2·6	57·3	2·6
	C (g per pot)	5·1	1·1	5·2	1·1
	b	1·00	0·11	1·01	0·11
100	ED_{50} (g ha^{-1})	4·95	0·84	4·61	0·62
0	r_2	0·63	0·11	0·52	0·08
80	r_3	1·16	0·22		
67	r_4	0·95	0·18		
50	r_5	0·76	0·14		
33	r_6	0·71	0·13		
20	r_7	0·57	0·11		
	$\eta_1 = \eta_2$			1·00	

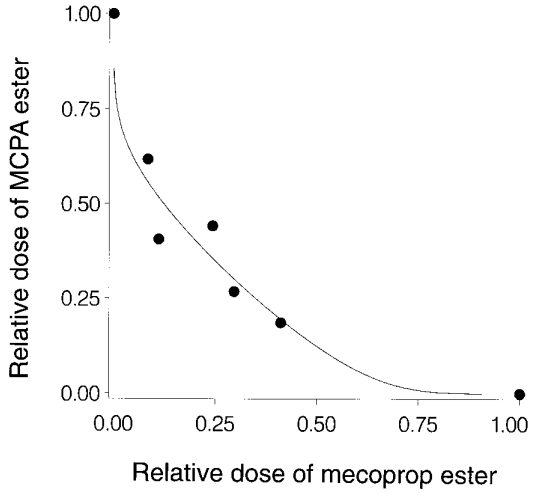

Fig. 5. Isoboles and data for ester formulations of MCPA and mecoprop-P from Table 4. The doses have been scaled so that the doses of MCPA and mecoprop-P applied separately are 1·00. (●) Mixture doses calculated from the parallel line regression in Table 4.

test for lack-of-fit[21] and by graphical analysis of the distribution of residuals.[10]

In Figs 2–5 the values for the mixtures, marked with black dots, were from the predicted values of the parallel regression analyses (eqn (2), the parameters of which are shown in Tables 2–4). Because the regressions within experiments were assumed mutually parallel, the positions of these mixture values are the same irrespective of the response level chosen. The isoboles are based upon the η_1 and η_2 from eqn (4) and shown as parameters in Tables 2–4.

3 RESULTS AND DISCUSSIONS

Table 2 shows a summary of the regression analyses for the tribenuron-methyl : mecoprop-P mixtures. Tests for lack of fit justified the assumption of parallel curves (Fig. 6), even though the two herbicides have different modes of action. The D and b parameters were virtually the same whether the parallel line assumption was used with or without incorporating eqn (4) with $\eta_1 \neq \eta_2$. There was no significant difference between models assuming $\eta_1 \neq \eta_2$, $\eta_1 = \eta_2$, $\eta_1 = 1 \neq \eta_2$ or $\eta_2 = 1 \neq \eta_1$, whereas ADM ($\eta_1 = \eta_2 = 1$) gave a significant test for lack of fit. Figure 2 shows the four isoboles. When $\eta_1 \neq \eta_2$, they have rather large standard errors which show that these parameters are not precisely determined. When assuming a common η, a symmetrical isobole is obtained with a reasonable precisely determined η, but the mixture values do not indicate symmetric isoboles. Also, when $\eta_1 = 1$ or $\eta_2 = 1$, then η_2 or η_1 are precisely determined. The pronounced antagonism with small proportions of mecoprop-P derived from eqn (2) was not significant in that it did not describe the data any better than with eqn (4) incorporated. The distribution of mixture ratios, however, was not optimal, in that the two ratios in the middle of the isoboles influenced the shape of the isoboles more than did the values with low proportions of mecoprop-P, and thus these few mixture ratios in the middle determined the isobole shape.

Strong antagonism has been demonstrated with mixtures of the dimethylamine salt of MCPA and chlorsulfuron or metsulfuron-methyl.[22] Kudsk and Mathiassen,[17] however, found that mixtures of mecoprop ethylene glycol diester and tribenuron-methyl followed ADM and Hollaway *et al.*[23] found synergistic effects with MCPA iso-octyl ester and sulfonylureas at ED_{75} and ED_{90} response levels. At ED_{50} level there was a tendency for the observed values to show a somewhat similar pattern to the one observed in Fig. 2. Undoubtedly, the formulation of the phenoxyalkanoic acid herbicides in mixtures with other herbicides may be important for the outcome of the mixture effects.[24]

Since MCPA (auxin herbicides) and tribenuron-methyl (inhibitor of acetolactate synthase) have different mode of action in the plant,[25] the MSM isoboles at

TABLE 4
Summary of Regression Analyses of Mixtures of Ester Formulations of MCPA (Reference Herbicide) and Mecoprop-P Using *Sinapis alba* as the Test Species

		Parallel curves		*Parallel curves and ADM*	
MCPA %	*Parameter*	*Estimate*	*Standard error*	*Estimate*	*Standard error*
	D (g per pot)	58·8	2·7	58·9	2·7
	C (g per pot)	—	—	—	—
	b	0·83	0·04	0·84	0·04
100	ED_{50} (g ha^{-1})	8·88	1·57	8·95	1·55
0	r_2	0·54	0·10	0·55	0·08
80	r_3	1·28	0·23		
67	r_4	1·59	0·29		
50	r_5	1·13	0·20		
33	r_6	1·21	0·21		
20	r_7	1·04	0·18		
	$\eta_1 = \eta_2$			0·27	0·14

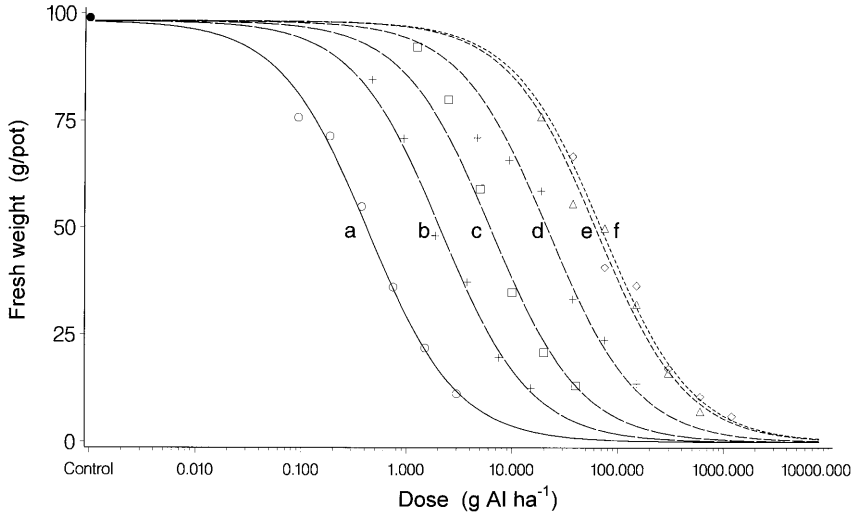

Fig. 6. Parallel dose-response curves for the mecoprop-P : tribenuron-methyl experiment from Table 2; a, b, c, d, e and f represent 0, 75, 92, 98, 99·5 and 100% mecoprop-P. Each value is the mean fresh weight of *Veronica persica* in a pot.

various response levels were also calculated (Fig. 3). Because the response curves were parallel the mixture values are the same irrespective of response level considered. Figure 3 shows that, at any response level, was MSM much worse in describing the isobole than was eqn (4). Drescher and Boedeker[16] pointed out in their work on relating ADM and MSM that, for small doses, the isoboles from ADM and MSM are practically indistinguishable, an observation which is confirmed here for the isobole marked c in Fig. 3.

Table 3 shows a summary of the regression analyses for the mixtures of salts of MCPA and mecoprop-P. The curves were mutually parallel and the 'best' fit was with ADM ($\eta_1 = \eta_2 = 1$). The mixture points, based upon the parallel regression in Table 3, deviated systematically from the ADM isobole (Fig. 4). The constraint by incorporating ADM in the eqn (2), however, did not result in significant test for lack of fit, which indicated that MCPA and mecoprop-P did not affect each other's action in the plant. In this case, the mixtures covered the isobole far better than in Fig. 2. Similar results have previously been found with root-absorbed phenoxyalkanoic acid herbicides, which were applied as technical grade compounds.[5]

Table 4 shows a summary of the regression analyses for the mixtures of ester formulations for MCPA and mecoprop-P. Also in this experiment, the parallel line assumption holds and the 'best' fit was that with $\eta_1 = \eta_2$. The isobole showed pronounced synergistic effects (Fig. 5) and the mixtures were well distributed along the isobole.

Because of their similar modes of action, we did not expect synergism between MCPA and mecoprop-P. It is therefore likely that the deviation from ADM for mixtures of the ester formulations is related to the effects of formulation constituents and not the active ingredients *per se*. Apparently, the ester formulations interact with each other, most likely in the absorption phase. This has recently been discussed by Hollaway *et al.*,[23] who found that the potency of a MCPA ester was not affected by a surfactant, whereas the same surfactant increased the potency of a MCPA amine. In a further example, Cabanne and Gaudry[26] demonstrated that the joint action of a mixture of aclonifen and bentazon depended upon whether the compounds were applied as technical grade materials or commercial formulations. The response curves were not parallel and therefore the deviation from ADM depended upon the response levels. Between ED_{20} and ED_{65}, mixtures of only active ingredients generally exhibited antagonism, whereas mixtures with formulated herbicides generally acted synergistically.[26]

Apparently, eqn (4) was able to describe isoboles, be it ADM or deviations from ADM. Two problems, however, are obvious from the results. The first problem is that mixture ratios must be evenly distributed along the isobole. This applies not only in order to get reliable estimates of η_1 and η_2 but also when we wish to draw the isoboles by hand. Finney[12] pointed out that unless *a priori* requirements govern the experimental design, the response curves for mixtures should be evenly spaced (based on $\log(ED_{50})$) between the response curves for the herbicides applied alone. If the mixtures are not properly distributed along the isobole, any conclusion about the shape of the isobole is debatable (see Fig. 2). The second problem is when η_1 and η_2 are different and both have to be estimated to obtain nonsymmetrical isoboles. η_1 and η_2 tend to be estimated with low precision, which may indicate over-parametrization or poor distribution of responses between the upper and lower limits.

It could be argued that the Multiplicative Survival Model (MSM),[3] being similar to the independent action,[14] is a better reference model for the mecoprop-P : tribenuron-methyl mixtures than ADM, but in this case MSM could not describe the joint action.

4 CONCLUDING REMARKS

Equation (4) is an attractive supplement to the widely used graphical presentations of isoboles,[7] because it combines statistical tests of the shape of isoboles. To get proper estimates of η_1 and η_2 with eqn (4), however, requires proper distribution of mixtures along the isobole. This requires knowledge of the relative potency between herbicides administered separately in order to design experiments with well-distributed mixture ratios.[4,12] The problem with wide standard errors of η_1 and η_2 should be investigated further. No doubt these parameters are prone to ill-defined mixture ratios, not covering the whole isobole and also illustrate the general problem of correlations between parameters in nonlinear regression.

ACKNOWLEDGEMENT

We wish to thank the Ministry of Food, Agriculture and Fisheries' Research Secretariat for financial support and Dr Aage Vølund for valuable comments.

REFERENCES

1. Flint, J. L., Cornelius, P. L. & Barrett, M., Analyzing herbicide interactions: A statistical treatment of Colby's method. *Weed Technol.* **2** (1988) 304–9.
2. Simpson, D. M. & Soller, E. W., Physiological mechanisms in the synergism between thifensulfuron and imazethapyr in sulfonylurea-tolerant soybean (*Glycine max*). *Weed Sci.*, **44** (1996) 209–14.
3. Morse, P. M., Some comments on the assessment of joint action in herbicide mixtures. *Weed Sci.*, **26** (1978) 58–71.
4. Green, J. M. & Streibig, J. C., Herbicide mixtures. In: *Herbicide Bioassays*, ed. J. C. Streibig & P. Kudsk. CRC Press, Boca Raton, 1993, pp. 117–35.
5. Streibig, J. C., Joint action of root-absorbed mixtures of auxin herbicides in *Sinapis alba* L. and barley (*Hordeum vulgare* L.). *Weed Res.*, **27** (1987) 337–47.
6. Green, J. M., Jensen, J. E. & Streibig, J. C., Models to assess joint action of pesticide mixtures. *Asp. Appl. Biol.*, **41** (1995) 61–8.
7. Gessner, P. K., Isobolographic analysis of interactions: an update on applications and utility. *Toxicology*, **105** (1995) 161–79.
8. Vølund, A., Dose response surface bioassay. *XVIth International Biometric Conference, Vol II*. Hamilton, New Zealand, 1992, p. 249.
9. Finney, D. J., *Statistical Method in Biological Assay*, 2nd edn. Charles Griffin & Company Ltd., London, 1978.
10. Streibig, J. C., Rudemo, M. & Jensen, J. E., Dose-response curves and statistical models. In: *Herbicide Bioassays*, ed. J. C. Streibig & P. Kudsk. CRC Press, Boca Raton, 1993, pp. 29–55.
11. Jerne, N. K. & Wood, E. C., The validity and meaning of the results of biological assays. *Biometrics*, **5** (1949) 273–99.
12. Finney, D. J., *Probit Analysis*, 3rd edn. Griffin, London, 1971.
13. Hewlett, P. S., Measurement of the potencies of drug mixtures. *Biometrics*, **25** (1969) 477–87.
14. Hewlett, P. S. & Plackett, R. L., *An Introduction to the Interpretation of Quantal Responses in Biology*, Edward Arnold, London, 1979.
15. Hewlett, P. S. & Plackett, R. L., A unified theory for quantal responses to mixtures of drugs: Non-interactive action. *Biometrics*, **15** (1959) 591–610.
16. Drescher, K. & Boedeker, W., Assessment of the combined effects of substances: the relationship between concentration addition and independent action. *Biometrics*, **51** (1995) 716–30.
17. Kudsk, P. & Mathiassen, S. K., Joint action of tribenuron and other broadleaf herbicides. *Asp. Appl. Biol.*, **41** (1995) 95–102.
18. Streibig, J. C., Joint action of root-absorbed mixtures of DPX-4189 and linuron in *Sinapis alba* L. and barley. *Weed Res.*, **23** (1983) 3–9.
19. Carroll, R. J. & Ruppert, D., *Transformation and Weighting in Regression*. Chapman and Hall, New York, 1988.
20. Box, G. E. P. & Cox, D. R., An analysis of transformations. *J. Royal Statistical Soc.*, **26** (1964) 211–52.
21. Seefeldt, S. S., Jensen, J. E. & Fuerst, E. P., Log-logistic analysis of dose-response relationships. *Weed Technol.* **9** (1995) 218–27.
22. Mathiassen, S. K. & Kudsk, P., Joint action of sulfonylurea herbicides and MCPA. *Weed Res.*, **33** (1993) 441–7.
23. Hollaway, K. L., Hallam, N. D. & Flynn, A. G., Synergistic joint action of MCPA ester and metsulfuron-methyl. *Weed Res.*, **36** (1996) 369–74.
24. Liu, S. H., Quick, W. A., Hsiao, A. I. & Streibig, J. C., Effect of MCPA on the phytotoxicity of imazamethabenz-methyl applied to wild oats (*Avena fatua* L.). *Weed Res.*, **34** (1994) 425–31.
25. Devine, M., Duke, S. O. & Fedtke, C., *Physiology of Herbicide Action*. PTR Prentice Hall, Englewood Cliffs, New Jersey, 1993.
26. Cabanne, F. & Gaudry, J. C., Effect of formulation agents on the joint action of aclonifen and bentazon. *Proc. Second Internat. Weed Control Cong. III*, ed. H. Brown *et al.* Dept of Weed Control and Pest. Ecology, Slagelse, 1996, pp. 881–6.